some, the identification of the HLA type is the first step towards the indirect identification of that person's sixth chromosome. If the HLA of the parents of that individual are known, it is generally easy to determine which HLA are derived from the maternal chromosome and which HLA are derived from the paternal chromosome. It therefore follows that if a child possesses no HLA in common with an alleged father, that man is excluded. But if certain HLA patterns are common to both the child and the alleged father the statistical likelihood of that man being the father can be determined. This statistic is influenced by how frequently the HLA patterns being shared are found in the general population. Because there are many HLA patterns, any single pattern tends to occur rarely and often a probability of paternity of 95 percent or greater is found. Despite this, we and others have experienced rare situations in which an alleged father could not be excluded by HLA testing but was successfully excluded by red-cell antigen inheritance patterns or isoenzymes. We therefore recommend that, in addition to HLA testing, as many erythrocyte and serum markers as possible be analyzed in the evaluation of possible paternity.

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Clinical Application of Free Cortisol Estimation

FROM TIME TO TIME the evaluation of a hypertensive patient raises the question of adrenal hyperfunction.

Excess adrenal corticosteroid secretion, Cushing's syndrome, is associated with truncal obesity, a florid appearance and hypertension. Many middle-aged obese individuals with no evidence of hormonal imbalance have a similar appearance. The laboratory can provide useful information to distinguish the two groups.

Cortisol is the major glucocorticoid secreted by the adrenal cortex in response to adrenocorticotropic hormone (ACTH) stimulation. About 90 percent of it is carried in the blood bound largely to an α-globulin called transcortin. The remainder occurs as physiologically active free cortisol. Bound cortisol acts as a reservoir for a supply of hormone available to the tissues of the body. Free cortisol is integrated into body metabolism and approximately 1 percent of it is secreted unaltered in the urine.

Plasma cortisol is a direct measure of bound plus unbound hormone. Diurnal variation normally occurs, with morning awakening levels approximately twice the 4 to 5 PM values. Because elevations of transcortin are associated with increased levels of cortisol, one anticipates elevated plasma cortisol in association with pregnancy or use of agents such as birth control medication.

Urinary free cortisol levels reflect the biologically active unbound hormone. The level is only loosely related to 17-hydroxycorticoids or 17-ketogenics, which are largely liver-derived metabolic breakdown products of cortisol. These derivatives vary with liver function, drugs affecting liver functions and cortisol levels. Excretion of 17-hydroxycorticoid varies with body size and thyroid status.

Urinary free cortisol does not have such interferences. It is one of the most reliable, informative tests to differentiate patients with endogenous Cushing's syndrome from normal persons or from those with central obesity, hirsutism and abdominal striae. Because the test is a specific radioimmunoassay there is no technical interference from drugs. The 24-hour urine sample averages out diurnal variations. Urinary free cortisol normal values are 20 to 100 μg per 24 hours.

An abnormally elevated test result should be followed by plasma cortisol estimations with dexamethasone suppression to clarify the comparative importance of the pituitary and adrenal glands in a patient's illness. The test is not useful for evaluating adrenal insufficiency.

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Hemapheresis

WITH THE ADVENT of modern technology, therapeutic bloodletting has attained a new aura of respectability. *Hemapheresis,* a term derived from the Greek word *aphairesis,* meaning "taking away," refers to procedures in which a component from donor or patient blood is selectively removed

and the remainder of the blood returned to the person.

The major improvement of the new technology has been in allowing selectivity of the component removed, and more specific terms such as plasmapheresis, leukapheresis and plateletpheresis reflect this selective removal.

The collection, component removal and return of the blood are performed by manual and semiautomated techniques, many of these involving differential centrifugation. These methods have greatly enhanced our ability to provide blood component support of platelets and granulocytes for patients undergoing aggressive chemotherapy.

Hemapheresis has also been used more directly on patients to treat a number of diseases, particularly those in which an abnormal protein, an autoimmune antibody or an antigen-antibody complex may play a role. However, only in the hyperviscosity syndrome is there broad agreement that this is the treatment of choice. Promising results have been seen in Goodpasture's syndrome and myasthenia gravis, but the actual contribution of plasmapheresis, drug therapy and supportive care have not yet been defined. In thrombotic thrombocytopenic purpura, the number of patients reported to survive seems to be increasing but, here again, the relative roles of supportive care, earlier diagnosis, drug therapy and plasmapheresis are unclear.

The use of plasmapheresis in the treatment of other conditions is even further from clear proof of efficacy. Recently the National Center for Health Care Technology reported on its evaluation of plasmapheresis in the treatment of rheumatoid arthritis. The report concluded that "plasmapheresis, lymphoplasmapheresis and lymphapheresis for rheumatoid arthritis should be considered experimental, with the possible exception of treatment for life-threatening complications of rheumatoid arthritis such as vasculitis, cryoglobulinemia or hyperviscosity syndrome."

So despite the continuing interest in possible applications of hemapheresis, many of the current applications must still be considered experimental. Most of the successful reports of therapeutic hemapheresis have involved only small numbers of patients.

Careful consideration of what therapeutic goals are desired and how to objectively assess their achievement is most important. These procedures are expensive and not innocuous. Fatal myocardial infarction has been reported to occur during hemapheresis, though no definite causal relation has been established. Increased risk of sepsis in immunosuppressed patients, rebound paradoxical increase in unwanted antibodies and anticoagulant-related citrate toxicity are some of the complications to be considered. The as-yet-unproved benefits of hemapheresis must be weighed against the potential and as-yet-uninvestigated, long-term and short-term ill effects, as well as the substantial health care costs.

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Value of the Muscle Biopsy

THE MAJOR REASON for doing a muscle biopsy is to elicit and confirm the presence of neuromuscular disease. In the past pathologists have been confined to evaluating the presence of denervation, myopathy or inflammation. With the advent of histochemistry, electron microscopy, immunohistochemistry and specialized biochemical procedures, pathologists are now able to evaluate neuromuscular disease from a broader perspective.

Physicians responsible for the clinical evaluation and care of patients with neuromuscular disorders must be in close communication with the pathologist before and after the biopsy. For optimal results a skilled surgeon interested in neuromuscular disorders should do the procedure.

To obtain maximum information, biopsy specimens must be properly processed, with portions soaked in isopentane cooled by liquid nitrogen for histochemistry, another portion processed for electron microscopy and fresh or frozen material stored for later detailed microchemistry, if needed. Such sophisticated procedures, equipment and interpretive expertise for the coordinated investigation of neuromuscular disease preclude routine evaluation of biopsy specimens in most community hospitals. However, we have found that with communication between the referring hospital staff and personal transport of a biopsy specimen (without freezing) within a half hour, excellent preservation can be obtained for histochemical and biochemical results.

Biopsy specimens undergo an enzyme histo-